

HERITABLE ASPECTS OF ANOMALOUS MYELINATED FIBRE TRACTS IN THE FOREBRAIN OF THE LABORATORY MOUSE

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Summary:

Serial coronal or sagittal sections were stained for myelin and examined in 6 inbred, 4 hybrid, and 2 outbred mouse strains. Absent corpus callosum was seen only in BALB/cJ as reported by Wimer, but a wide range of the size of corpus callosum was also noted. The action of a major gene was not evident in backcross or F₂ generations; polygenic and perhaps epistatic inheritance was indicated. In A/J, and to a lesser extent A/HeJ and BALB/cJ, the columns of fornix frequently collided with the anterior commissure and either passed around it to make a normal termination or deflected dorsally to make an abnormal termination in lateral septum. In some BALB/cJ brains the anterior commissure instead was displaced and passed behind or through the columns of fornix. Backcross and F₂ data suggested inheritance, was polygenic and that genetic variation affected the spatio-temporal coordination of ontogeny of the two tracts. Finally, unusual longitudinal bundles were detected in the septal region of BALB/cJ. Results of crosses were consistent with the hypothesis that a single, incompletely dominant gene was acting, but further study of both the anatomy and heredity of the defect was deemed necessary.

Article:

INTRODUCTION

Although an impressive number of deleterious mutant genes which modify nervous system structure and function have been detected in the mouse^{27,34}, genetic variation in the brains of laboratory strains commonly employed in behavioural research has received scant attention. Systematic evaluations of the brains of several inbred rat and mouse strains have revealed large genetic variation in brain weight^{12,28,38}, volume of neocortex and hippocampus⁴³, and concentration of several presumed neurotransmitters and related enzymes^{16,29,32}. Success in selectively breeding for high and low brain weight⁶ and high and low brain ChE levels³⁰ has been achieved, and complementary changes in brain weight^{11,31,36}, ACh, and AChE³¹ as a correlated response to selection for maze learning ability have been observed.

Studies have also been conducted of organizational variation within precisely delimited brain regions in various strains. Differences in the topographical distribution of optic nerve terminations in the lateral geniculate and anterior colliculus have been reported for albino and pigmented strains of several mammalian species^{3,8,9,13,23}, although differences have not been proven to be caused by the albino gene itself. Genuine organizational variation within an outbred mouse strain has even been detected⁴⁴.

The present paper reports genetic variation which lies between the gross level of whole brain or regional size and the finer level of synaptic organization. A variety of unusual courses of myelinated fibre tracts in the mouse forebrain are described, and their modes of inheritance are examined. Based upon these findings, hypotheses about mechanisms of gene actions in neurogenesis are suggested which possess implications for genetic differences in adult brain and behaviour.

MATERIALS AND METHODS

Some of the anomalies described herein were originally detected serendipitously in a study of spatial location of major fibre tracts in 7 laboratory mouse strains⁴L. Since the defects were rather striking, a number of other commercially available strains were also examined, and appropriate cross-breeding was undertaken to examine the mode of inheritance of the anomalies. Several relevant characteristics of the mice employed in the present study are presented in Table 1. All inbred and hybrid mice were procured from the Jackson Laboratory, Bar Harbor, Maine, while out-bred mice were obtained from Carworth Farms, New City, N.Y., and Simonsen Laboratories, Gilroy, Calif. Crossbred generations were propagated and raised in the author's laboratory at Waterloo. All but a very few mice were 76-93 days of age, a time during which the size and structure of the mouse brain are very stable¹⁹.

Routine histological procedures for myelin staining were used to facilitate processing of large numbers of brains. Each mouse was given a lethal dose of Nembutal and was then perfused intracardially with 0.9 % saline followed by 10% formalin. After extraction and weighing, some brains were encased in 10% gelatin, while others remained in formalin until sectioning. For some brains (see Table 1), serial, coronal sections were taken on a freezing microtome at 25 μ m, saving every other section, while other brains were cut sagittally at 33 μ m, saving every third section. All sections were stained with Sudan Black B, a simple stain for the phospholipids in myelin sheaths; myelin appeared brilliant blue-green against a clear background.

TABLE I
CHARACTERISTICS OF MICE USED IN STUDY

Strain	Supplier	Coat colour	Age range	Number of Brains	
				Coronal	Sagittal
<i>Inbred</i>					
A/J (139)*	Jackson	Albino	77-78	10	3
A/HeJ (141)	Jackson	Albino	85	10	-
BALB/cJ (100)	Jackson	Albino	46-141	23	28
C57BL/6J (95)	Jackson	Black	76-78	10	-
C57L/J (108)	Jackson	Steel gray	83-86	10	-
DBA/2J (93)	Jackson	Dilute brown	76-78	10	-
<i>F₁ hybrid</i>					
B6AF ₁ /J (C57BL/6 × A)**	Jackson	Black	77-78	9	-
B6D2F ₁ /J (C57BL/6 × DBA/2)	Jackson	Black	77-79	10	-
CAF ₁ /J (BALB/c × A)	Jackson	Albino	51-87	10	11
LAF ₁ /J (C57L × A/He)	Jackson	Brown	85-91	10	-
<i>Outbred</i>					
CF1	Carworth	Albino	78-79	10	-
SWISS	Simonsen	Albino	77-78	10	-
<i>Crossbred</i>					
B6AF ₁ /J × A/J	Waterloo	Several	79-81	20	56
CAF ₁ /J × BALB/cJ	Waterloo	Albino	79-80	-	49
CAF ₁ /J × CAF ₁ /J	Waterloo	Albino	79-93	-	62

* Generations of brother by sister inbreeding as of 1967¹⁰.

** Parent strains with strain of female parent given first.

Evaluation of the major fibre tracts in each brain was conducted with the assistance of the excellent atlas of the C57BL/61 mouse brain by Sidman *et al.*³³. Particular attention was devoted to tracts of the forebrain, for this region was the site of most anomalies originally observed. Criteria for an anomaly were generally that it should be prevalent in only a few strains and that it should not occur in the prototypical C57BL/6J mouse, an inbred animal of reproductive distinction and consequent low cost. As will be evident, these criteria were sufficiently broad to include one 'defect' with no genetic basis at all. It should also be emphasized that all observations were made by the author. Hence, any anomalies detected are probably exceedingly gross, and a plethora of other deviations will undoubtedly appear under the scrutiny of a more perceptive observer using more sensitive histological methods.

Nomenclature follows the Latin according to Sidman *et al.*³³, with an occasional English equivalent. Abbreviations for several fibre tracts (Table II) are generally consistent with those of König and Klippel²⁰ for the rat brain.

RESULTS AND DISCUSSION

At least 4 major types of anomalies were observed, each of which existed in varying degree and variety within certain strains. Since the modes of inheritance of these defects were found to be quite different, each anomaly is analysed separately below.

TABLE II

STRUCTURES AND THEIR ABBREVIATIONS*

C	Cingulum	FR	Fasciculus retroflexus
CAA	Commissura anterior, pars anterior	GCC	Genu corporis callosi
CAE	Capsula externa	PCVT	Plexus choroideus ventriculi tertii
CAP	Commissura anterior, pars posterior	RCC	Radiatio corporis callosi
CC	Corpus callosum	SM	Stria medullaris thalami
CCA	Commissura colliculorum anteriorum	STC	Stria terminalis, pars commissuralis
CFD	Commissura fornicis dorsalis	TCC	Truncus corporis callosi
CFV	Commissura fornicis ventralis	TD	Tractus diagonalis(Broca)
CH	Commissura habenularum	THC	Tractus hippocampocorticalis
CO	Chiasma opticum	TOC	Tractus olfactocorticalis
CP	Commissura posterior	TOI	Tractus olfactorius intermedius
F	Columna fornicis	TSHT	Tractus septohypothalamicus
FLC	Fissura longitudinalis cerebri	TST	Tractus septotubercularis
FMI	Forceps minor	VL	Ventriculus lateralis
FMP	Fasciculus medialis prosencephali	VT	Ventriculus tertius
FMT	Fasciculus mammillothalamicus	II	Nervus opticus
FO	Fornix	sl	Nucleus septi lateralis
FPC	Fornix precommissuralis		

* Adapted from König and Klippel²⁰.

Multiple bundles of commissura anterior, pars anterior

During routine analysis of brains of several mouse strains, it was noticed that the commissura anterior, pars anterior (CAA) was sometimes composed of several smaller bundles instead of a single large bundle showing intense, continuous myelination. It was further observed that the multiple bundles were sometimes relatively small ($< 85 \mu\text{m}$) satellite bundles to the main CAA, while in other instances the main CAA in each hemisphere was divided into 2 or 3 large bundles (each $> 85 \mu\text{m}$). Examples of brains either with no multiple bundles or with both small and large bundles are shown in Fig. 1.

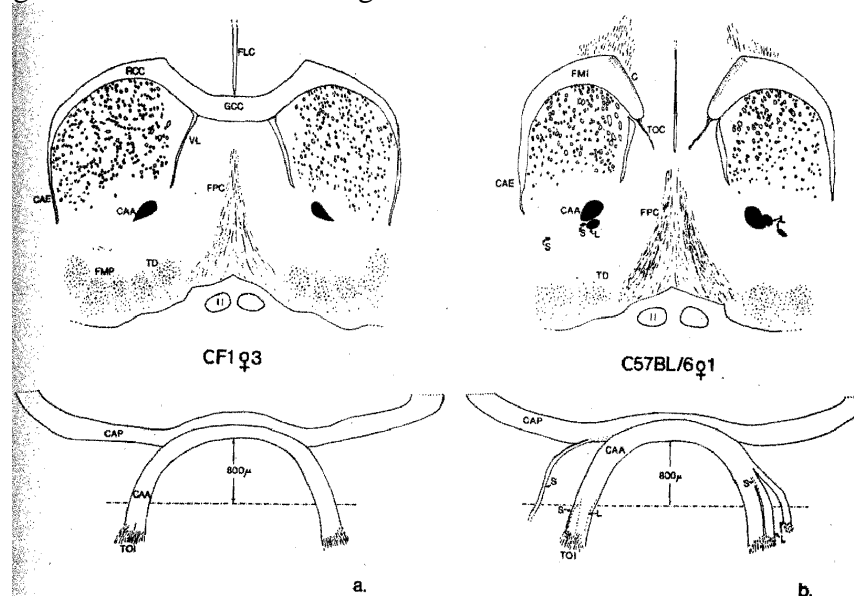


Fig. 1. Examples of a brain with no multiple bundles of CAA (a, CF1) or with several extra bundles of CAA (b, C57BL/6). Small bundles are indicated as S, and large ones are labelled L. Drawing of the coronal section was taken from a section $800 \mu\text{m}$ from the midline crossing of CAA. Horizontal representations of CAA and CAP were reconstructed from tracings of serial coronal sections. The level from which the coronal drawing was taken is shown by a dotted line in the horizontal reconstruction. See Table II for abbreviations used in this and other figures.

The extent of these multiple bundles in the 12 inbred, hybrid and outbred strains listed in Table I was assessed by counting the total number of small and large bundles for each brain from serial coronal sections. Only bundles which appeared in at least 3 successive sections (length of 150 μm) were included. No significant differences in total bundles were detected between groups ($F = 1.6$, $df = 11/107$, $P > 0.10$). All groups showed an excess of bundles beyond the ideal of a single compact bundle on each side, the mean number of excess bundles being 3.3. Of the 119 brains evaluated, only 3 had but a single major bundle in each hemisphere. No significant correlations between numbers of bundles in left and right hemispheres within the same brain were found.

In view of these results, the tendency of CAA to divide into several bundles appears to be a normal occurrence in the mouse brain. It is worthy of note that similar bundles also appear in Fig. 23 of Sidman *et al.*³³, and in Fig. 2 of King¹⁷. Thus it is clear that a characteristic may be bizarre without being truly anomalous in the genetic sense.

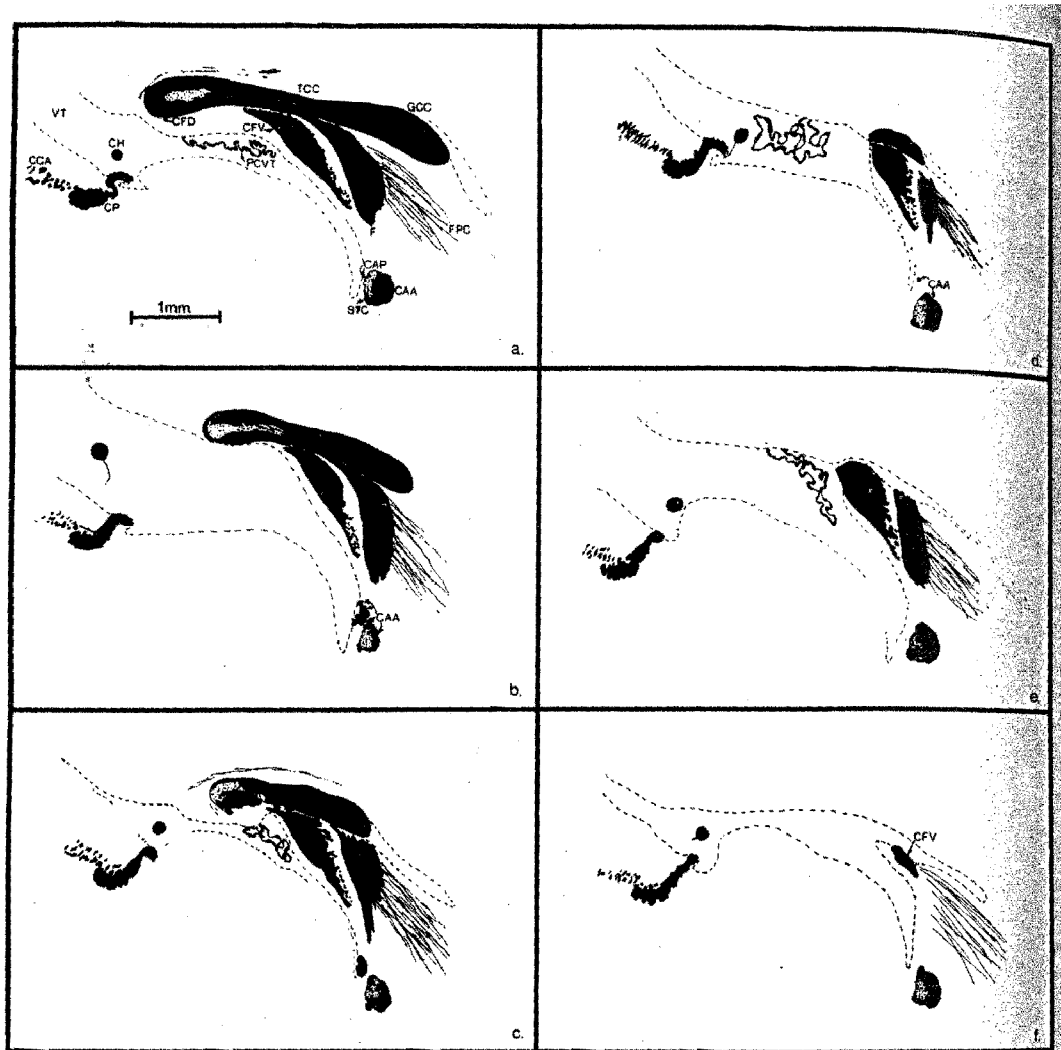


Fig. 2. Drawings of mid-sagittal sections for 6 different BALB/cJ brains. a: corpus callosum is well within the normal range of variation. All other structures are normal. b: corpus callosum is short (2.51 mm), but it is still considered normal. Several bundles of displaced CAA are evident. c: corpus callosum (1.87 mm) which is just below the cutoff point for normal length. A large displaced bundle of CAA also occurs. CFD is present. d: corpus callosum is very short (0.68 mm), and CFD is absent. A small bundle displaced from CAA is shown. e: corpus callosum and CFD are absent, but other structures are normal. f: corpus callosum is absent, and CFV is greatly diminished. Columns of fornix (F) are displaced laterally in this brain and therefore do not appear in the mid-sagittal region.

Deficient corpus callosum

Absence of corpus callosum (CC) in the mouse has been attributed to a single gene (*ac*) with total penetrance by Keeler for a stock of mice which has since become extinct^{15,18,34}. However, a later detailed report by King¹⁷ on the anatomical features of the defect stated that 'The genetic aspects have not as yet been fully worked out' (p.

362); comments in that report suggested that the penetrance of the proposed gene was in fact less than complete. A similar defect of CC has recently been detected in inbred mouse strains BALB/cJ and 129/J⁴². Unlike the earlier results, full expression of absence of CC appeared in only about 20% of BALB/cJ mice. Since the BALB/cJ strain has been inbred for over 100 generations¹⁰, whatever locus may produce the defect is likely to be homozygous, indicating partial penetrance of the presumed mutant gene.

In view of these uncertainties about the heredity of absent CC, the mode of inheritance of the defect in BALB/cJ was studied further. Examination of the 12 strains in Table I confirmed that only BALB showed any gross defect of CC. The degree of defect was quantified by measuring the maximum length of CC from the beginning of the genu to the end of the splenium at the mid-sagittal level. It was evident that a wide range of expression of CC was present in BALB mice. Examination of forebrain fibre tracts in the vicinity of CC at midline with reference to the degrees of defect described by King¹⁷ revealed no instances of CC present posteriorly but not anteriorly, while all cases of short CC were found to be of the type located dorsally to commissura fornicis ventralis (CFV). A surprising defect which occurred in two mice with totally absent CC was absence or great reduction of CFV! Examples of several degrees of defect are sketched in Fig. 2.

Mode of inheritance of deficient CC was ascertained using segregating F₂ and backcross generations derived from BALB/cJ and A/J parent strains. Lengths of CC in the F₁ hybrid between BALB and A (CAF₁/J) were entirely normal (Fig. 3), indicating recessive inheritance. The hypothesis that deficient CC was caused by a single, recessive gene with partial but constant penetrance was first evaluated. The degree of penetrance was estimated to be 39.2% in BALB using the criterion of a defective CC as one less than 2.0 mm long (see Fig. 3). Since the proportion of hypothesized recessive genotypes in an F₂ cross should be 0.25, the proportion of aberrant CC phenotypes should be 0.25×0.39 or 0.098. Using the number of F₂ offspring (Table 1), the expected number of CC deficiencies in F₂ is 6. However, as shown in Fig. 3, no defective CCs were observed in F₂, which contradicts the single gene hypothesis ($\chi^2 = 5.6$, $df = 1$, $P < 0.025$). In the F₁ \times BALB backcross the expected proportion of recessive genotypes is 0.50, and the expected proportion of deficient CC is 0.196. Nonetheless, only one of 49 backcross offspring exhibited deficient CC, and the single gene hypothesis was again contradicted ($\chi^2 = 10.7$, $df = 1$, $P < 0.005$). The one backcross individual which was defective had no CC at all, but CFV was intact.

Although the data certainly oppose the simple idea that deficient CC is the result of a single gene for which BALB/cJ is homozygous, alternative interpretations involving the presence of a major gene cannot be entirely excluded. It is conceivable that the BALB/cJ strain is currently segregating at a locus affecting CC size, even though full-sib matings are being used. This possibility is unlikely, since the frequency of absent CC has not changed appreciably subsequent to 1965 when Wimer first reported the defect, but it can be tested by additional genetic analysis within the BALB/cJ strain. It is also possible that the penetrance of the gene is higher on homozygous than on heterozygous backgrounds. Finally, a maternal influence mediating a major gene effect may exist, since hybrid mothers were used to obtain all segregating generations. All of the above interpretations can be tested only by obtaining litters from BALB/cJ females, a thankless task indeed owing to their very poor breeding qualities. Nonetheless, such research is being undertaken in the author's laboratory to clarify the mode of inheritance.

Further insight into the inheritance of CC size can be gained by examination of variation within the normal range. In the F₁ mice from the C57BL/6 \times A cross the length of CC significantly exceeded the midparent value ($F = 10.3$, $P < 0.005$), indicating dominance. The F₁ \times A backcross scores, however, did not reveal any hint of a bimodal distribution as would be expected if a major gene were present; in fact, the modal backcross length was very rare in either parent strain or their hybrid. Backcross scores did not deviate significantly from the mean of F₁ and A ($F < 1.0$). Thus, the length of CC in these strains is most likely influenced by a large, undetermined number of genes with primarily additive actions and no epistasis.

In the cross involving A and BALB, on the other hand, F₁ scores were far in excess of those of either parent strain. This also occurred in the backcross to BALB, even when the one mouse with no CC was ignored.

Although F_2 and backcross means did not differ, the variability within the backcross population was significantly greater than within F_2 ($F = 2.7$, $df = 47/60$, $P < 0.0005$), and there were more mice with relatively long CC in $F_1 \times$ BALB than in F_2 ($P < 0.05$, Kolmogorov—Smirnov 2-sample test³⁵). These results present the interesting possibility that BALB/cJ genes have the potential to form a large CC but that they are not so expressed in the homozygous state. This notion is strengthened by the observation that the brain weights of BALB/cJ mice are larger than those of any other strains employed in this study.

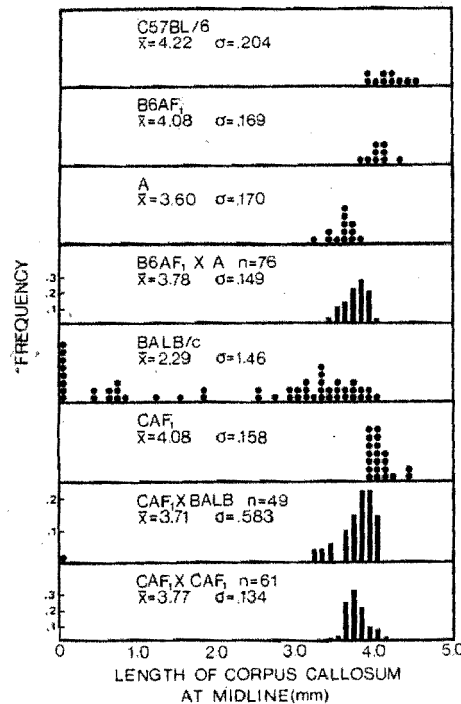


Fig. 3. Length of the corpus callosum from the tip of the genu to the tip of the splenium measured from a tracing of a mid-sagittal section. Means and standard deviations are shown for each group. Dots represent individual subjects, while solid bars represent the proportions of subjects at each length in groups with many subjects.

In conclusion, the length of CC in BALB/cJ mice reveals the presence of two distinct genetic phenomena. The first one, absence or gross deficiency of CC, occurs primarily in a highly homozygous state, and even then it is only an occasional event. In these respects it strongly resembles a phenodeviance, as discussed by Lerner^{5,22}. The most cogent explanation for such an anomaly is that an unfavourable set of genes in the homozygous condition disrupts the normal buffering of the growth process, and developmental homeostasis is lost. When homeostasis is regained by cross-breeding to achieve heterozygosity, a second genetic process affecting CC length is revealed that is polygenic and non-epistatic. If this is true, then the BALB/cJ strain must possess the potential for a much larger CC than is actually observed.

Although a number of models are conceivable which posit that a specific combination of alleles at several loci disrupts the formation of CC, the hypothesis that the homozygous background leads to unstable development in BALB is currently more parsimonious in view of the great variability seen both within and between BALB/cJ mice for other phenotypes. Large variability in BALB compared to other inbred or even outbred strains has been noticed for brain weight^{38,41}, heart rate², audiogenic seizure topography²¹, and morphology of the external genitalia⁴⁰. Furthermore, BALB mice become pregnant with great difficulty and are often exceedingly poor mothers, which indicates severe inbreeding depression^{5,22}.

Stray bundles of columna fornicis and CAA

At least 4 distinct types of unusual myelinated fibre bundles have been observed to deviate from the prototypical pattern of fornix and columna fornicis normally seen in C57BL/6J mice. Each type will be described briefly, and evidence pertaining to the mode of inheritance will be presented.

The columna fornicis (F) normally pass posterior to commissura anterior (CAA and CAP) and proceed in two symmetrical, well-circumscribed bundles to the nuclei of the mammillary bodies. However, in certain mice part of one or both bundles of F left the main bundle just dorsal to CAA and then travelled around the anterior aspect of CAA (see Fig. 4a). In all cases of this nature, the stray F bundles eventually rejoined the F proper prior to their terminations, although this reunion sometimes did not occur until all bundles were in the mammillary bodies proper. To verify these observations of myelin-stained sections, 3 A/J mice were subjected to electrolytic lesions of F in the medial septal area and were then sacrificed after 1 week. A modified Fink—Heimer stain for degenerating fibres and terminals²⁴ confirmed that the stray fibres all rejoined F; no abnormal terminations were detected. The frequencies of mice of different strains showing this type of anomaly are shown in Table HI under the heading 'F loops around CAA'.

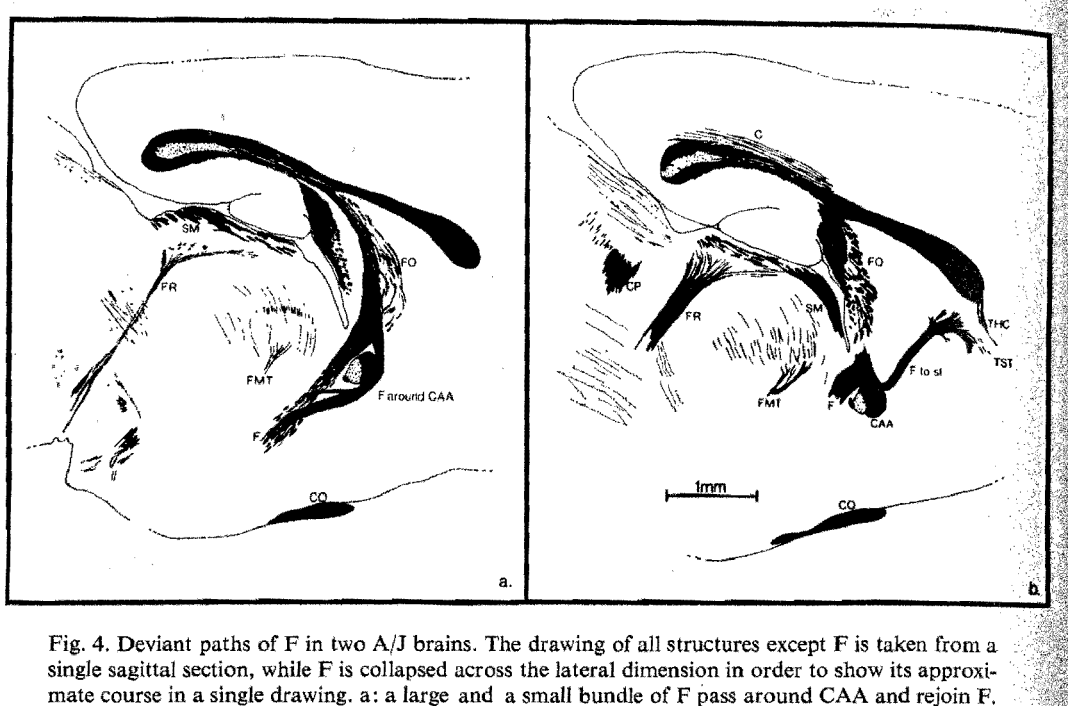


Fig. 4. Deviant paths of F in two A/J brains. The drawing of all structures except F is taken from a single sagittal section, while F is collapsed across the lateral dimension in order to show its approximate course in a single drawing. a: a large and a small bundle of F pass around CAA and rejoin F. b: a large bundle of F collides with CAA, travels into lateral septum (sl) and disperses.

In other mice part of F also left the main bundle and made contact with the dorsal surface of CAA, but the fibres instead turned abruptly and proceeded dorsally through the lateral septal area (see Fig. 4b). The courses of these fibres were highly variable, but their myelin-staining properties always disappeared within the limits of the lateral septal area. The bundles often made one or more unusual turns before vanishing. Evidence of their terminations from the Fink—Heimer staining was inconclusive because the degenerating F fibres reentered the area of lateral septum (sl) destroyed by the lesion. These defects are listed as 'F loops to sl' in Table III.

A larger number of mice showed a less severe sort of F deviation in which a bundle of F left the main bundle, travelled laterally along the dorsal surface of CAA, and then made a precise loop back to F. Where these bundles terminated could not be ascertained. In some instances a bundle appeared to enter the fornix (FO), although it may have gone through FO in order to reach F. At any rate, these fibres were classified as 'F loops to F or FO'.

The inheritance of all 3 types of loop emanating from F appeared to be polygenic, for the frequencies observed in all 3 segregating crosses departed from predictions based upon a single gene model. Results from the B6AF₁ × A backcross were especially clear, since the parent strains (C57BL/6 and A) showed extreme values. Fewer F loops were seen in the backcross than were expected for a single gene character. Loops which did appear were generally much smaller bundles than those observed in the A/J strain, although a few backcross mice had anomalies as extreme as the least affected A/J mice. Closer examination of serial, coronal sections suggested a simple hypothesis to account for observed differences. When the distances between the first appearance of FO and the first point at which CAA crossed midline were compared, highly significant strain differences were

evident ($\omega^2 = 0.52$, $F = 18.9$, $P < 0.0001$). The mean separations of the two structures in the anterior—posterior dimension were 255 μm , 195 μm , 122 μm and 15 μm for A, $F_1 \times A$, F_1 , and C57BL/6, respectively. Thus, a more anterior location of FO with respect to CAA was correlated with a greater frequency of anomalous bundles involving F, which suggests that the spatial location of two structures determines whether one fibre tract will collide with and perhaps grow around another. The F_1 separation did not differ from the mean of A and C57 ($F < 1.0$), and the backcross mean did not differ from the mean of A and F_1 ($F < 1.0$); hence, additive or non-epistatic inheritance was indicated. The relative spatial locations of the two structures also appeared to have a polygenic basis, since no evidence of segregation was seen in the distribution of backcross scores.

It remains to be determined why some stray F bundles pass around CAA or loop back to F and then continue to their proper destination while others grow dorsally into lateral septum. This latter event is definitely an abnormal situation, and it justly deserves the appellation 'polygenic catastrophe', at least in the anatomical sense.

TABLE III

PERCENT OF SUBJECTS SHOWING EACH TYPE OF ANOMALY

Group	N	Displaced CAA	F Loops			FO through sl		Other	Any	Mean anomalies per brain
			Around CAA	To F or FO	To sl	Short	Long			
A/J	13	0.	84.6	84.6	69.2	30.8	0.	30.8	100.	6.4
A/HeJ	10	0.	40.0	80.0	30.0	20.0	10.0	90.0	100.	3.8
BALB/cJ	51	78.4	25.0*	50.0**	30.0**	30.0**	92.2	80.0**	100.	10.3
C57BL/6J	10	0.	0.	0.	0.	0.	0.	0.	0.	0.
C57L/J	10	0.	0.	0.	0.	0.	0.	0.	0.	0.
DBA/2J	10	0.	0.	0.	0.	0.	0.	0.	0.	0.
B6AF ₁ /J	9	0.	0.	11.1	0.	0.	0.	11.1	11.1	0.2
B6D2F ₁ /J	10	0.	0.	0.	0.	0.	0.	0.	0.	0.
CAF ₁ /J	21	4.8	28.6	85.7	23.8	14.3	23.8	47.6	100.	4.8
LAF ₁ /J	10	0.	0.	0.	0.	0.	0.	0.	0.	0.
CF1	10	0.	10.0	0.	0.	0.	0.	10.0	20.0	0.2
SWISS	10	0.	0.	0.	0.	0.	0.	0.	0.	0.
CAF ₁ \times BALB	49	14.3	38.8	83.7	20.4	14.3	63.3	16.3	95.9	5.4
CAF ₁ \times CAF ₁	62	4.8	40.3	85.5	22.6	14.5	30.6	16.1	98.4	4.3
B6AF ₁ \times A	76	1.3	29.0	63.2	25.0	5.3	0.	17.1	82.9	2.2

* Based on 28 brains.

** Based on 10 brains, all with normal corpus callosum.

Further evidence that the inheritance is additive as opposed to epistatic was present in the observations of CAF₁, the hybrid cross of BALB/c and A. Both parent strains exhibited F loops, and CAF₁ was affected to a roughly comparable degree. Little else could be said about the inheritance in crosses involving BALB genes, since an accurate assessment of frequencies of defects was not possible in BALB because of the presence of other major defects such as absent CC or displaced CAA.

Although the commissura anterior, pars anterior (CAA) usually crosses midline as a single large bundle of myelinated fibres, mice of the BALB/cJ strain were often observed to have bundles of CAA displaced from the major bundle. Deviations from the normal path occurred primarily in the vicinity of F, and the deviant bundles usually rejoined CAA before it turned anteriorly towards the olfactory bulbs. In Many cases the stray CAA bundles passed either through or behind F (see Fig. 2). The degree of anomaly in BALB mice varied from a small bundle displaced from the dorsal surface of CAA nearest F (Fig. 2d) to almost the entire CAA displaced through and behind F (Fig. 2b). These deviations are referred to as 'displaced CAA' in Table III.

Displaced CAA occurred with greatly reduced frequency and severity in all crosses involving BALB. Careful inspection of the F and CAA bundles in collision suggested at least two causes of displaced CAA. First, the spatial relationships of the two fibre tracts were such that a collision of some sort was likely, much as was shown for the A and C57BL/6 crosses. Second, the temporal order of arrival of the two tracts appeared to be different in A and BALB during ontogeny. Whereas F always grew around CAA in the A strain, sometimes

CAA clearly grew around F in BALB. although F was also observed to pass around a displaced CAA bundle in some instances. If it can be assumed that a bundle which deviates from a direct path in order to pass around another bundle in the adult brain actually arrived after the immovable bundle during ontogeny, then the observations suggest that CAA forms before F arrives in A mice, while CAA and F arrive at approximately the same time in BALB mice. The only study of time of appearance of tracts in the mouse forebrain indicated that F and CAA arise sometime between 16.5 days and 18.5 days¹⁴, but their actual temporal order of appearance is not known. The variety of interactions in BALB also suggests that the timing of growth of the two tracts is quite variable, a supposition which is quite consistent with the hypothesized disruption of developmental homeostasis in BALB. When BALB and A are crossed, the delay in time of arrival of F relative to CAA imposed by the additive action of A strain genes is presumably sufficient to make displaced CAA a rather rare occurrence. Backcrossing to BALB raises the frequency of displaced CAA to a somewhat higher level than the F₁ or F₂ frequency. Thus, both 'displaced CAA and 'F loops around CAA' are believed to result from improper temporal coordination as well as spatial location of the growing F and CAA. The genetic bases of both spatial and temporal aspects of their growth are believed to be nonepistatic and polygenic, and hence the aberrant paths taken by colliding fibres are threshold characters resulting from extreme spatio-temporal relationships.

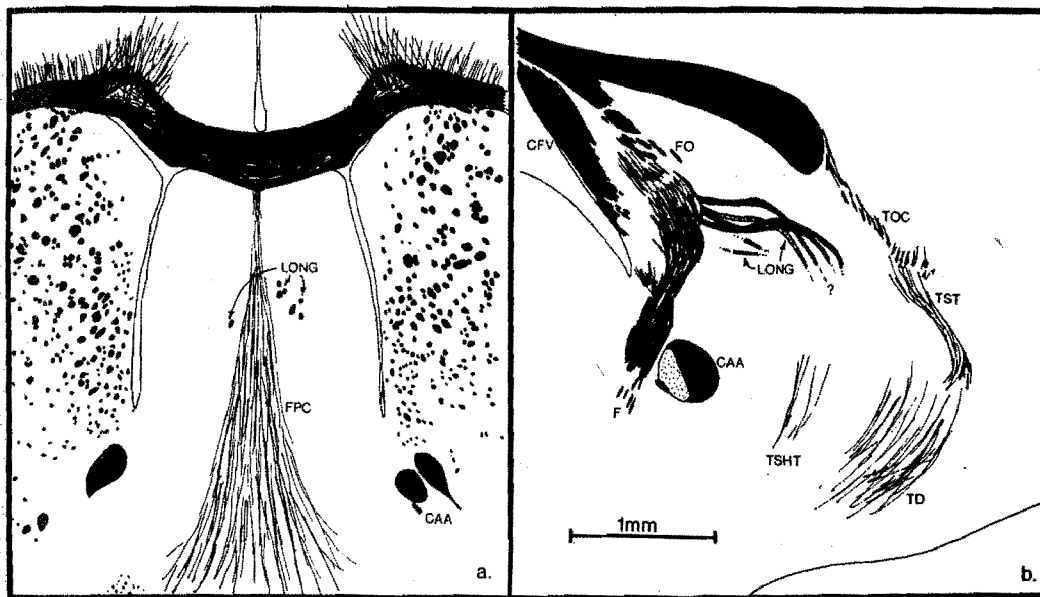


Fig. 5. Unusual myelinated fibres ('LONG') in two BALB/cJ brains. a: coronal section shows 7 longitudinal bundles in the medial septal area near FPC. b: sagittal section shows 6 longitudinal bundles travelling from FO through *sl*. The 'LONG' bundles are drawn from a composite of several consecutive sagittal sections, while the normal tracts appear in a single section.

Longitudinal bundles in lateral septum

A very unusual kind of fibre appeared in the septal area of BALB and related mice. Anywhere from 1 to 8 heavily myelinated bundles coursed directly from the FO in the medial septa] area (Fig. 5a) and travelled anteriorly about 1 mm and a varying distance laterally, at which point they usually turned in a ventral direction and dispersed (see Fig. 5b). The sites of their termination are unknown. It is unlikely that they represented large bundles of fornix precommissuralis, because they generally passed through the lateral septal area (*sl*) and then came close to one portion of CAA. They are termed 'FO through *sl*, LONG' in Table III.

The unusual longitudinal fibres in the septal area of BALB appeared to be the manifestation of a single, incompletely dominant gene. Using the frequencies of 'LONG' for A, BALB and CAF₁ given in Table III, expected frequencies were derived for F₂ and backcross generations. In neither segregating generation were observed frequencies of 'LONG' significantly different from predictions of a single gene hypothesis (both $\chi^2 < 1$, $P > 0.50$). Those F₂ and backcross offspring which were affected showed full expression of the longitudinal fibres. The unitary nature of the defect was also supported by the fact that only mice related to BALB were affected, with the exception of a single A/HeJ mouse. However, polygenic models are also consistent with these data, so additional genetic and anatomical evidence is needed before the gene can be named and

catalogued.

Other weird things

A number of other wayward bundles were detected less frequently (Table III), Some clearly involved FO, but they disappeared in the lateral septa] area after a short distance CFO through *sl*, SHORT'). Others were small and difficult to follow for more than two or three sections, They are designated 'OTHER'. Since these anomalies constituted an obscure and heterogeneous category, it is not surprising that their inheritance proved to be uninteresting.

Correlations among defects

If the various anomalies discussed above are indeed distinct characters with different genetic bases, then the presence or absence of a defect of one kind should not influence the occurrence of another type in the same brain. Pearson correlations between degrees of various anomalies within strains or crosses revealed that most anomalies were statistically independent; very few correlations reached acceptable levels of significance ($P < 0.01$). Three instances of correlations with 'LONG' bundles were noted. In $CAF_1 \times CAF_1$, brains with more 'LONG' bundles also showed greater frequency of 'F around CAA' ($r = 0.64$, $P < 0.001$) and 'F to *sl*' ($r = 0.33$, $P < 0.01$), while in $CAF_1 \times BALB$ more 'LONG' bundles were correlated with more 'F around CAA' ($r = 0.32$, $P = 0.025$). Comparisons between groups, however, showed that 'LONG' bundles were more frequent in $CAF_1 \times BALB$ than in $CAF_1 \times CAF_1$, whereas frequencies of unusual F bundles did not differ between groups (Table III). Thus, the correlations within groups were difficult to interpret.

No correlations between any anomaly and sex of the subject or coat colour (in $B6AF_1 \times A$ only) were significant.

GENERAL DISCUSSION

The present results are illustrative of several major categories of genetic effects upon brain structure.

- (a) No genetic effect: Multiple bundles of CAA were found to a similar extent in 12 inbred, hybrid, and outbred mouse strains.
- (b) Simple Mendelian inheritance: Longitudinal bundles from FO through *s/l* in BALB/cJ mice were inherited in a manner consistent with the hypothesis of a single gene with partial penetrance and incomplete dominance. However, further research on the anatomy and heredity of the anomaly is certainly needed.
- (c) Polygenic, non-epistatic inheritance: Both the length of corpus callosum at midline and the relative spatial positions of FO and CAA were found to conform closely to expectations of a polygenic model in crosses involving C57BL/6J and A/J. Inheritance was generally intermediate, and distributions of phenotypes were unimodal and approximately normal. A corollary polygenic effect, additive inheritance with a threshold, was evident in the collision of F and CAA in A/J and BALB/cJ strains; if F was located too far anteriorly with respect to CAA, one tract might collide with or even pass around the other.
- (d) Polygenic, epistatic inheritance: Deficiency or absence of corpus callosum in BALB/cJ was believed to be a polygenic character, and it apparently depended more upon the poorly-buffered development of the BALB strain than upon a specific gene carried by BALB for size of corpus callosum. However, additional study to evaluate alternative major-gene models is needed.

It should be emphasized at this point that it will be quite difficult to provide definitive evidence for the presence or absence of a major gene effect on brain structure when complete penetrance is lacking and when parent strains differ only in the degree or magnitude of a measure. The presence of epistatic interaction in which the expression of a gene at one locus depends upon the nature of genes at other loci also renders further study especially difficult. Nonetheless, these variations in the mouse brain are interesting and warrant further study. The relatively mild natures of the defects which render them resistant to simple genetic analysis also place them

within the normal range of variation in the mouse brain, unlike deleterious mutant genes which are quite obvious in their expressions but whose victims are clearly beyond the normal range of behavioural variation. The pertinence of these findings to well-known strain variation in behaviour remains to be demonstrated. Both the A/J and BALB/cJ strains are characterized by extreme levels of activity³⁷, aggression³⁷ and learning rate³⁹, but it is difficult to imagine how unusual courses of myelinated fibres could underlie such vast behavioural differences, especially when one considers that most deviations involved only a small part of a major bundle and that most deviant bundles rejoined the major bundle prior to its termination.

Perhaps the most interesting implications of these anomalous bundles are for the developmental processes from which they arise. As discussed previously, spatiotemporal variables in development may account for the unusual paths of certain fibres in brains of certain genotypes. Observations of neuronal maturation and axon growth in the developing embryo will provide important evidence relating to these issues. In addition, there is a good possibility that the study of genetic variation in the dynamics of neural development will reveal even more interesting differences in topographical organization. Whereas point-to-point connectionist ideas of brain organization have enjoyed considerable credibility in the past, recent evidence suggests that gradients of neural maturation may play an important part in determining brain structure. If the topographical distribution of interconnections between two brain regions depends in part upon the maturational state of one region when axons from the other arrive, then it is easy to imagine how genetic variation in the relative times of maturation of the two regions could yield genetic differences in topographical organization as well as in the routes of interconnecting fibre bundles. Similar considerations would apply to genetic variation in spatial relations between two regions; if the regions matured at the same relative times in brains of two genotypes, organizational differences would nonetheless exist if the regions were further apart in one genotype than in the other, since fibres growing at the same rate would arrive later if they had to grow further in one genotype.

These speculations about genetic variation in brain are made more plausible by studies of organizational plasticity in both the developing and the adult brain. If temporary connections may be made with non-preferred sites as a normal phase of neurogenesis^{1,25}, and if reorganization can occur following damage to the adult brain^{24,26}, then it is reasonable to suspect that genetic variation can also influence the degree of innervation of one brain region by another and that a fibre bundle displaced by collision with a more mature bundle might make an unusual but functional termination in a different region (*e.g.*, F to *sl*, Fig. 4b).

Of course, this discussion in no way confutes the idea of specific biochemical substances as cues for axon growth and synaptogenesis; such messengers are undoubtedly important in guiding the major fibre bundles to their destination through an informationally chaotic maelstrom of squirming axons and pulsating neuroblasts. Developmental gradients of maturation are most likely important in affecting organization within major regions, once the growing fibres have reached the general vicinity. Unlike mutations which may drastically modify the entire course of a major tract, genetic changes in spatio-temporal gradients may bring about intraregional changes in innervation which result in behavioural changes that nonetheless remain within the normal range of viable variation.

Thus, the anomalies of major forebrain fibre tracts are believed to constitute only a small subset of the actual genetic variation in the mouse brain, which, if studied at the proper level of complexity, will eventually reveal the neural substrates for genetic variation in behaviour.

REFERENCES

- 1 ALTMAN, J., AND DAS, G. D., Postnatal changes in the concentration and distribution of cholinesterase in the cerebellar cortex of rats, *Exp. Neural.*, 28 (1970) 11-34.
- 2 BLIZARD, D. A., AND WELTY, R., Cardiac activity in the mouse: strain differences, *J. comp. physiol. Psychol.*, 77 (1971) 337-343.
- 3 CREEL, D. J., AND GIOLLI, R. A., Retinogeniculo-striate projections in guinea pigs: albino and pigmented strains compared, *Exp. Neural.*, 36 (1972) 411-425.
- 4 EDDS, M. V., BARKLEY, D. S., AND FAMBROUGH, D. M., Genesis of neuronal patterns, *Neurosci. Res.*

Progr. Bull., 10 (1972) 254-367

- 5 FALCONER, D. S., *Introduction to Quantitative Genetics*, Ronald Press, New York, 1960, pp. 264- 275,330-337,
- 6 FULLER, J. L., AND GEILS, H. D., Brain growth in mice selected for high and low brain weight, *Develop. Psychobiol.*, 5 (1972) 307-318.
- 7 Gorruen, D. I., AND COWAN, W. M., Evidence for a temporal factor in the occupation of available synaptic sites during the development of the dentate gyrus, *Brain Research*, 41 (1972) 452-456.
- 8 Ginta,ERy, R. W., An abnormal retinogeniculate projection in the albino ferret, *Brain Research*, 33 (1971)482-485.
- 9 GUILLERY, R. W., AND KAAS, J. H., A study of normal and congenitally abnormal retinogeniculate projections in cats, *J. comp. Neurol.*, 143 (1971) 73-100.
- 10 GREEN, E. L. (Ed.), *Handbook on Genetically Standardized fax Mice*, The Jackson Laboratory, Bar Harbor, Maine, 1968, p. 4.
- 11 HAMILTON, J. A., *The Association between Brain Size and Maze Ability in While Rats*, Unpublished Ph.D. Diss., Univ. of California, 1935.
- 12 HENDERSON, N. D., Brain weight increases resulting from environmental enrichment : A directional dominance in mice, *Science*, 169 (1970)776-778.
- 13 KALIL, R. E., JHAVERI, S. R., AND RICHARDS, W., Anomalous retinal pathways in the Siamese cat; An inadequate substrate for normal binocular vision, *Science*, 174 (1971) 302-305.
- 14 KALLEN, B., The embryology of the telencephalic fibre systems in the mouse, *J. Embryol. exp Morph.*, 2 (1954) 87-100.
- 15 KEELER, C. E., Absence of the corpus callosum as a mendelizing character in the house mouse. *Proc. nat. Acad. Sri. (Wash.)*, 19 (1933) 609-611.
- 16 KESSLER, S., CIARANELLO, R. D., SHIRE, J. G., AND BARCHAS, T. D., Genetic variation in activity of enzymes involved in synthesis of catecholamines, *Proc. nat. Acad. Sci. Wash.*, 69 (1972) 2448- 2450.
- 17 KING, L. S., Hereditary defects of the corpus callosum in the mouse, *Mus musculus, J. comp. Neurol.*, 64 (1936) 337-363,
- 18 KING, L. S., AND KEELER, C. E., Absence of corpus call osum, a hereditary brain anomaly of the house mouse. Preliminary report, *Proc. nat. Acad. Sci. (Wash.)*, 18 (1932) 525-528.
- 19 KonAvAsm, T., INMAN, O., BUNO, W., AND HIMWICH, H. E., A multidisciplinary study of changes in mouse brain with age, *Recent Advanc. biol. Psychiat.*, 5 (1963) 293-308.
- 20 KONio, J. F. R., AND KLIPPEL, R. A., *The Rat Brain*, Williams and Wilkins, Baltimore, Md., 1963.
- 21 LEECH, C. K., *Rate of Development of Electrically Kindled Convulsions Compared to Audiogenic Seizures and Learning Ability in Seven Mouse Strains*, Unpublished Ph.D. Diss., Univ. of Waterloo, Waterloo, Ontario, Canada, 1972.
- 22 LERNER, I. M., *Genetic Homeostasis*, Dover, New York, 1970,134 pp.
- 23 Luisa), R. D., Uncrossed visual pathways of hooded and albino rats, *Science*, 149 (1965) I506-I507.
- 24 LYNCTA, G., MATTHEWS, D. A., MOSICO, S., PARKS, T., AND COTMAN, C., Induced acetylcholinesterase-rich layer in rat dentate gyrus following entorhinal lesions, *Brain Research*, 42 (1972) 311-318.
- 25 MARIN-PADILLA, M., Prenatal ontogenetic history of the principal neurons of the neocortex of the *cat (Felix domestica)*. A Golgi study. II. Developmental differences and their significances, *Z. Anat. Entwickl.-Gesch.*, 136 (1972)I25-142.
- 26 MOORE, R. Y., BSORKURRIO, A., AND STENEVI, U., Plastic changes in the adrenergic innervation of the rat septal area in response to denervation, *Brain Research*, 33 (1971) 13-35.
- 27 *Mouse News Letter*, 47 (1972) 3-15.
- 28 PADEH, B., *A Genetic Analysis of Operant Discrimination Learning and Brain Size in Inbred and Heterogeneous Strains of Laboratory Mice*, Unpublished Ph.D. Diss., Univ. of Colorado, 1972.
- 29 PRYOR, G. T., SCHLESINGER, K., AND CALHOUN, W. H., Differences in brain enzymes among five inbred strains of mice, *Life Sci.*, 5 (1966) 2I05-211I.
- 30 RODERICK, T. H., Selection for cholinesterase activity in the cerebral cortex of the rat, *Genetics*, 45 (1960) 1I23-1I40.

- 31 ROSENZWESO, M. R., Effects of heredity and environment on brain chemistry, brain anatomy, and learning ability in the rat, *Kansas Stud. Educ.*, 14 (1964) 3-15.
- 32 SCHLESINGER, K., AND GRIM, B. J., The genetics and biochemistry of audiogenic seizures. In G. LINDZEY AND D. D. THIE.SSEN (Eds.), *Contributions to Behavior-Genetic Analysis. The Mouse as a Prototype*, Appleton-Century-Crofts, New York, 1970, pp. 219-257.
- 33 SIDMAN, R. L., ANGEVINE, J. B., AND TABER PIERCE, E., *Atlas of the Mouse Brain and Spinal Cord*, Harvard Univ. Press, Cambridge, Mass., 1971.
- 34 SIDMAN, R. L., GREEN, M. C., AND APPEL, S. H., *Catalog of the Neurological Mutants of the Mouse*, Harvard Univ. Press, Cambridge, Mass., 1965.
- 35 SIEGAL, S., *Nonparametric Statistics*, McGraw-Hill, New York, 1956, pp. 127-136.
- 36 SILVERMAN, W., SHAPIRO, F., AND HERON, W. T., Brain weight and maze learning in rats, *J. comp. Psychol.*, 30 (1940) 279-282.
- 37 SOUTHWICK, C. H., AND CLARK, L. H., Interstrain differences in aggressive behavior and exploratory activity of inbred mice, *Commun. Behav. Biol.*, 1 (1968) 49-59.
- 38 STORER, J. B., Relation of lifespan to brain weight, body weight, and metabolic rate among inbred mouse strains, *Exp. Gerontol.*, 2 (1967) 173-182.
- 39 WAHLSTEN, D., Genetic experiments with animal learning: A critical review, *Behav. Biol.*, 7 (1972) 143-182.
- 40 WAHLSTEN, D., Unpublished observations.
- 41 WAHLSTEN, D., HUDSPETH, W. J., AND WEENING, D. L., Variability within and between several mouse strains in the spatial location of major fibre tracts of the brain, Unpublished manuscript.
- 42 WIMER, R. E., Personal communication.
- 43 WIMER, R. E., WIMER, C. C., AND RODERICK, T. H., Genetic variability in forebrain structures between inbred strains of mice, *Brain Research*, 16 (1969) 256-264.
- 44 WOOLSEY, T. A., Somatosensory, auditory and visual cortical areas of the mouse, *Johns Hopis. Med. J.*, 121 (1967) 91-112.